# Document Title
Medium Lot to Lot Testing JA2

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## Control Information
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- **Owner:** WATE02
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STCL-SOP-052 JA2
MEDIUM LOT-TO-LOT TESTING

1 PURPOSE
1.1 Methylcellulose based medium (MethoCult) and Iscove’s Modified Dulbecco’s media (IMDM) are the standard gelling agent and preparation media used in Colony Forming Cell assays (CFC) in the Stem Cell Laboratory (STCL). When a new lot number arrives, the lab must verify the quality and evaluate the performance of these reagents before they are put into use.

2 INTRODUCTION
2.1 Each batch of MethoCult and IMDM is sterility tested by Stem Cell Technologies. The quality control of performance of MethoCult is also tested in CFC assays using human Bone Marrow (BM), Cord Blood (CB), HPC-Apheresis (HPC-A) also known as peripheral stem cells (PSC), or Peripheral Blood (PB) samples by the manufacturer. The STCL performs internal quality control on new lots of media to ensure reproducibility of the colonies enumerated. New lot numbers of media are compared to the current lot number (that is in use) by plating the same specimen using each lot. The plates are read after the 14-16 day incubation period and the colony counts compared against one another.

3 SCOPE AND RESPONSIBILITIES
3.1 The Medical Director, Laboratory Manager, QA Manager, and applicable STCL staff are responsible for ensuring the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS
4.1 CFC Colony Forming Cell
4.2 STCL Stem Cell Laboratory
4.3 BM Bone Marrow
4.4 CB Cord Blood
4.5 HPC-A Hematopoietic Progenitor Cell Assay
4.6 PSC Peripheral Stem Cells
4.7 PB Peripheral Blood
4.8 HPCA Hematopoietic Progenitor Cell Assay
4.9 QA Quality Assurance
4.10 HPC Hematopoietic Progenitor Cell
4.11 IMDM Iscove's Modified Dulbecco's Media
4.12 SOP Standard Operating Procedure
4.13 CO2 carbon dioxide
5 MATERIALS

5.1 Reagents:

5.1.1 MethoCult 4434 Medium  Stem Cell Technologies/ Cat# 4434
5.1.2 IMDM with 2% FBS  Stem Cell Technologies/ Cat# 7700
5.1.3 Sterile water 1L  Sigma/ Cat# W3500-1L

5.2 Supplies:

5.2.1 3 ml sterile syringes with luer lock tip  BD Biosciences/ Cat# 309657
5.2.2 Blunt-end needle, 16G  Stem Cell Technologies/ Cat# 28110
5.2.3 24-well Costar cell culture plates  Corning Inc./ Cat# 3524
5.2.4 Sample ID barcodes  CompuType/ Cat# 1018184
5.2.5 Permanent marker  Sharpie/ Cat# 30001
5.2.6 Sterile 12 x 75 polystyrene tubes  Port City Diagnostics/ Cat# T2063STR
5.2.7 Sterile 15 ml conical tubes  Port City Diagnostics/ Cat# 1100SRGFT
5.2.8 Sterile 200μl pipette tips  Port City Diagnostics/Cat# 7509-96RS
5.2.9 Sterile serological pipettes  Port City Diagnostics/ 1ml Cat# SER-010-S01
5.2.10 Do Not Use label
5.2.11 This Lot is Ready for Use label

2ml Cat#SER-0020-S01
5ml Cat#SER-0050-S01
10ml Cat#SER-0100-S01

6 EQUIPMENT

6.1 Barcode scanner  Zebra/ Model ZM400
6.2 Inverted Microscope  Olympus IMT-2
6.3 Thermo Scientific CO2 Incubators  HERAcell 150 SN# 225658 & SN# 225659
Isotemp Plus SN# Z01J464990ZJ
VWR/ Mini Vortexer MV1
6.4 Vortex Mixer
6.5 DIFFCOUNT electronic cell counter  Modulus Data Systems/ SN# 319806
6.6 Stamping Clock  Latham 1000E/ SN# 1E014032
6.7 3 channel traceable timer  Fisher Scientific/ SN# 111878606 / SN# 111878753
6.8 Class II Biological Safety Cabinet  NUAIRE 425-600/ SN# 123044050508
Baker SG400/ SN# SL29877V
6.9 Micropipettes  Rainin/ 20μl : SN# M11689G
20μl: SN# 10985053K
200μl: SN# 10984537K
200μl: SN# M10263E
7 SAFETY

7.1 All procedures for cell processing and set-up of cell culture assays should be performed using sterile technique under a biohazard safety cabinet certified for Level II handling of biological materials, and universal handling precautions. When handling a biological hazardous substance, such as umbilical cord blood, appropriate personal protective equipment (PPE), must be worn as the primary barrier of protection. PPE may include, but is not limited to: face protection, lab coats and gloves. Appropriate PPE should be donned before handling potentially hazardous biological materials and removed immediately and replaced if gross contamination of the equipment occurs.

8 PROCEDURE

8.1 Record the NEW lot number information on the HPC Reagent Verification Log. If it is a new lot number, place a "DO NOT USE" red sticker on each bottle and store the media at -20°C, as per manufacturer’s recommendations.

8.2 When ready to perform the lot-to-lot verification, thaw a bottle of media from the new lot number according to manufacturer’s directions (See MethoCult Technical Manual).

8.3 Aliquot thawed media into 15 ml sterile conical tubes and label each tube with name of reagent, lot number, aliquot date, technologist’s initials, and expiration date.

8.4 Prepare a 24 well culture plate.

8.5 Prepare two syringes and two tubes of the MethoCult and/or IMDM, one with the current lot and one with the new lot number.

8.6 Select a sample (CB, PSC, BM, or PB) and plate according to the SOP; using the same plate, plate one sample using the current lot number(s) of media and the other sample using the NEW lot number(s) of media. Label each sample on the plate accordingly.

8.7 Place the plate in a 37°C humidified 5% CO₂ incubator for 14-16 days.

8.8 Enumerate the colonies for each sample and record the results on the Progenitor Assay Form. Place a copy of that form in the HPC Reagent Verification Log.

8.9 Since there are factors that could contribute to the variability of the results obtained from this assay (i.e., equipment and/or culture conditions, integrity of the sample being used, etc.), the total colony count, between lots, should not exceed 20%.

8.10 If the criterion is NOT met, repeat the lot-to-lot comparison using a new sample; document this failure and subsequent corrective action on the HPC Reagent Verification Log.

8.11 If the criterion IS met, results of this comparison will be recorded on the Media Lot to Lot QA Release Log and reviewed by the QA or lab manager to approve/release the lot for use. Record the “IN USE” date on the Reagent Verification Log. Replace the “DO NOT USE” red labels with “THIS LOT READY FOR USE” green labels.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-SOP-052 HPCA – Cord Blood Bank Products
9.2 STCL-SOP-052 (JA1) – HPC Processing of Fresh UCB Samples – Flow Chart
9.3 STCL-SOP-052 (FRM1) Progenitor Cell Assay Form (Cord Blood Products)
9.4 STCL-PROC-022 (FRM 1) Hematopoietic Progenitor Cell Assay Sheet
9.5 STCL-PROC-022 (FRM 2) Media Lot-to-Lot QA Release Log
9.6 STCL-PROC-022 (FRM 3) Reagent Verification Log
9.7 STCL-PROC-022 JA1 Tech Manual HPCA - MethoCult

10 REFERENCES
10.1 Human Colony-Forming Assays Using MethoCult, Stem Cell Technologies, Version 4.0.0, April 2012

11 REVISION HISTORY

<table>
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<tr>
<th>Revision No.</th>
<th>Author</th>
<th>Description of Change(s)</th>
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| 03           | Barbara Waters-Pick | • Added “Do NOT Use” labels to Supply Section 5.2.10  
• Added “This Lot is Ready for Use” labels to Supply Section 5.2.11  
• Added Section 11 Revision History to track modifications to this job aide |
## Signature Manifest

**Document Number:** STCL-SOP-052 JA2  
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All dates and times are in Eastern Time.

### STCL-SOP-052 JA2 Medium Lot to Lot Testing JA2

#### Author

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#### Manager

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